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Research Article

Cell surface characterization of *Yarrowia lipolytica* IMUFRJ 50682

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Abstract

In the present work, the surface characteristics of a wild-type strain of *Yarrowia lipolytica* (IMUFRJ50682) were investigated. Six different methods to characterize cell surfaces — adhesion to polystyrene; hydrophobic interaction chromatography (HIC); microbial adhesion to solvents (MATS) test; zeta potential; microbial adhesion to hydrocarbons (MATH) test; and contact angle measurement (CAM) — were employed to explain the cell surface behaviour of *Y. lipolytica* (IMUFRJ50682). This *Y. lipolytica* strain presents significant differences at the cell surface compared with another *Y. lipolytica* strain (W29) previously reported in the literature. The main difference is related to the higher cell adhesion to non-polar solvents. The proteins present on the cell wall of *Y. lipolytica* IMUFRJ50682 seem to play an important role in these particular surface characteristics because of the consistent reduction of this yeast hydrophobic character after the action of pronase on its cell wall.

Keywords: ***Yarrowia lipolytica*; cell surface; hydrophobic substrates; surfactant**

Introduction

Yarrowia lipolytica is an unique, strictly aerobic yeast with the ability to produce a wide spectrum of products, such as organic acids, extracellular enzymes, etc. It is considered non-pathogenic and several processes based on this organism were classified as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA), USA (Barth and Gaillardin, 1997). It is one of the most extensively studied 'non-conventional' yeasts and is currently used as a model for the study of protein secretion, dimorphism, degradation of hydrophobic substrates and several other fields (Fickers *et al*., 2005).

Y. lipolytica's growth and metabolite secretion are affected by different environmental factors (Corzo and Revah, 1999). The amount of oxygen available to this microorganism seems to be an important parameter (Alonso *et al*., 2005; Kamzolova *et al*., 2003). Recently, the addition of perfluorocarbons (PFCs) to the culture medium of *Y. lipolytica* was reported as a novel approach to enhance the oxygen uptake (Amaral *et al*., 2006a). The oxygen permeability on the PFC is much higher than in water with the solubility being 10–20 times higher in PFC than in water. Amaral *et al*. (2006a) also observed a curious partition of the yeast between the aqueous and organic PFC phase, with an unexpected preference of the yeast for the organic solvent. These peculiar interactions may be related to the ability of this microorganism to degrade hydrophobic substrates (Barth and Gaillardin, 1997).

The growth of microorganisms on a hydrophobic substrate (HS) requires the transport of the HS from the organic phase to the cell surface and the contact between the HS and the cell.

This contact can occur through a direct adsorption of hydrophobic droplets to the cell surface, or it can be mediated by a surfactant. In fact, with *Y. lipolytica*, evidence of both mechanisms has been reported in literature. This yeast has been reported to produce surfactants during growth on HS (Cirigliano and Carman, 1984, 1985) and microscopic observations have revealed the attachment of methyl ricinoleate to its surface (Aguedo *et al*., 2003).

In the case of direct adsorption, several mechanisms can be involved, including hydrophobic, Lewis, electrostatic or van der Waals interactions and some tests have been developed to characterize the hydrophobic properties of microorganisms (Bellon-Fontaine *et al*., 1996; Mozes and Rouxhet, 1987; Rosenberg, 1981; van der Mei *et al*., 1995). The aim of this work is to explain the preference of *Y. lipolytica* IMUFRJ 50682 for the PFC organic phase by means of a cell surface characterization using techniques previously proposed in the literature. The investigation of a surfactant production in the culture medium of this *Y. lipolytica* strain is the subject of another article (Amaral *et al*., 2006b).

A number of assays to characterize cell surfaces based on the adhesion of cells to hydrocarbons (van der Mei *et al*., 1995), solvents (Bellon-Fontaine *et al*., 1996) and surfaces (Rosenberg, 1981) have been proposed. It has been argued (van der Mei *et al*., 1998) that these methods essentially probe an interplay of physicochemical and structural factors involved in microbial adhesion, rather than one single factor, e.g. the cell surface hydrophobicity. Contact angle measurements have been recognized as the universal standard for microbial cell surface hydrophobicity (van der Mei *et al*., 1998), especially if combined with isoeletric point (IEP) values determined by zeta potential measurements (Rijnaarts *et al*., 1995). However, the cell adhesion assays also provide important information related to the cell surface and its interaction with different materials or substances, especially by using different methods together to complement each other. Therefore, we have gathered the information obtained from several of these methods to characterize the cell surface of *Y. lipolytica* IMUFRJ 50682.

Materials and methods

Materials

Perfluorodecalin, whose common brand name is Flutec PP6, was obtained from Flutec. The relevant physical properties of perfluorodecalin at 25 °C and 1 atm are as follows: density 1.917 g/ml; vapour pressure 810 Pa and oxygen solubility 127.8 mg/l (Dias *et al*., 2004). Pronase, phenyl sepharose CL 4B, *n*-hexadecane, toluene, hexane, chloroform and ether ethyl were purchased from Sigma. Peptone, yeast extract and glucose were obtained from Merck, Oxoid and Isofar, respectively.

Strain, media and culture conditions

A wild-type strain of *Yarrowia lipolytica* (IMUFRJ 50682) was selected from an estuary in the vicinity of Rio de Janeiro, Brazil (Haegler and Mendonça-Haegler, 1981) and conserved at 4 °C on YPD-agar medium. Cells from a preculture (cultivated for 48 h in YPD medium at 160 r.p.m.), in stationary growth phase, were used to inoculate 500 ml shake flasks containing 200 ml medium for cell growth for 170 h. For both preculture and cell growth, cells were cultivated at 28 °C in a rotary shaker (160 and 250 r.p.m., respectively) in flasks containing YPD medium (w/v: yeast extract, 1%; peptone (from casein), 0.64%; glucose, 2%). For experiments using perfluorocarbon, 20% v/v perfluorodecalin was added to the medium. A control experiment was carried out with no perfluorocarbon.

Yarrowia lipolytica W29 (ATCC20460; CLIB89) was provided by the Biological Engineering Centre from Universidade do Minho (Braga, Portugal). This strain was used to compare the surface characteristics of these two different strains of the same species.

Methods to characterize the cell surface

Preparation of samples

For all methods used to characterize the cell surface, the collection and preparation of samples was performed as follows: cells from the preculture or from the growth medium were harvested ($3000 \times g$, 10 min), washed twice with distilled water and resuspended in the buffer or solution determined by the method in question. This procedure was performed in order to assure the complete

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removal of any substance that was not covalently linked to the cell surface, in particular, a possible surfactant.

Adhesion to polystyrene

This test is based on the assumption that there is a correlation between the hydrophobicity of the cells and their adherence to polystyrene, as proposed by Rosenberg (1981). A 1 ml volume of a cell suspension in 0.1 M potassium phosphate buffer at pH 3.0, 5.0, 7.0 or 9.0 and at a fixed concentration was poured into a polystyrene Petri dish and left to settle for 24 h. The supernatant was then removed by the insertion (10 times) of the dish in a 2 l Becker with 1.5 l deionized water agitated at 1000 r.p.m. After 2 h the dish was observed under the microscope to quantify the cell adhesion using image analysis.

Hydrophobic interaction chromatography (HIC)

The test consists in measuring the amount of cells retained by a hydrophobic gel (Mozes and Rouxhet, 1987). Phenyl sepharose CL 4B, bed volume approximately 0.6 ml, was contained in a Pasteur pipette and equilibrated with a solution of 4 M NaCl at a certain pH (3–7), fixed by 0.1 M citrate buffer. A cell suspension was prepared in the same solution, which served for equilibrating the gel. A sample of 0.1 ml of the cell suspension was introduced on the gel, and 3 ml of the equilibrating solution was allowed to pass through the column and the OD_{570} of this fraction was considered as A_0. A few portions of buffer, 3 ml each, at the same pH value as the equilibrating solution but with no NaCl, were then passed through the column and their OD_{570} values were noted as A_i. In addition, 0.1 ml of the original cell suspension was diluted in 3 ml 4 M NaCl, which provided the value of A_t by measuring its OD_{570}. The data allow the determination of the portion of cells retained by the gel at high ionic strength ($R_H = 100^*(A_t - A_0)/A_t$), which is favourable to the observation of hydrophobic interactions and the portion of cells which remain retained in conditions of lower ionic strength ($R_L = 100^*(A_t - \Sigma A_i)/A_t$).

MATS test

Lewis acid–base (i.e. electron donor–electron acceptor) interactions were evaluated using the MATS method (Bellon-Fontaine *et al*., 1996). This technique is based on the microbial affinity to a monopolar and to an apolar solvent. The monopolar solvent can be acidic (electron acceptor) or basic (electron donor), but both solvents must have similar surface tension Lifshitz–van der Waals components. The solvent pairs used were chloroform (electron acceptor) and hexadecane and ethyl ether (electron donor) and hexane.

Cells were resuspended in a phosphate buffer (0.1 M, pH 7) to an optical density at 570 nm (OD_{570}) = 0.70. This cell suspension (3.6 ml) was vortex-mixed for 50 s with 0.6 ml solvent. After 10 min, the aqueous phase OD_{570} was measured. Results are given as percentages of bound cells: % adhesion $= 1 - A/A_0$, where A_0 and A are OD_{570} of the aqueous microbial suspension before and after mixing, respectively. The hydrophobicity (Hyph) in this method is defined as the cell adhesion to hexadecane. The electron donor character (EDC) is obtained by the cell adhesion to chloroform minus that to hexadecane, and the electron acceptor character (EAC) is the cell adhesion to ethyl ether minus that to hexane.

Zeta potential

The electrokinetic zeta potential was studied on a Coulter Delsa 440SX-type instrument. Measurement was performed with cells harvested by centrifugation at 2000 × *g* for 10 min and suspended in aqueous solution of KCl (0.1 M).

MATH test

Since the original microbial adhesion to hydrocarbons (MATH) test has been criticized for not being sufficiently quantitative, due to the neglect of kinetic effects, the so-called 'kinetic MATH test' described by van der Mei *et al*. (1995) has been here employed at room temperature.

The buffers used were: PUM (19.7 g/l K_2HPO_4, 7.26 g/l KH_2PO_4, 1.8 g/l H_2NCONH_2 and 0.2 g/l $MgSO_4 \cdot 7H_2O$); PBS (0.87 g/l K_2HPO_4, 0.68 g/l KH_2PO_4 and 8.77 g/l NaCl); and KPi (0.87 g/l K_2HPO_4, and 0.68 g/l KH_2PO_4).

Cells were resuspended in a buffer (PUM, PBS and KPi; different pHs) to $OD_{570} = 0.60$. This cell suspension (3 ml) was vortex-mixed for 10 s with 0.15 ml hydrocarbon (hexadecane or toluene). After 10 min, the aqueous phase OD_{570} was measured. The latter procedure was repeated with

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different vortexing time (V_t) and $\log(A/A_0{}^*100)$ (where A_0, and $A - OD_{570}$ of the aqueous microbial suspension before and after mixing, respectively) was plotted against V_t. Linear least square fitting subsequently yielded the initial removal rate, R_0 (per min) as a measure of the adhesion of cells to the hydrocarbon, i.e. of the hydrophobicity by MATH.

Pronase treatment

Yeast cells, harvested by centrifugation and washed twice, were suspended in 0.01 M Tris–HCl buffer to give $OD_{570} = 10$. Pronase was added to the cell suspension at a concentration of 0.1 mg/ml, and the cells were harvested after incubation for a determined period of time in a shaking water bath at 37 °C (Tae-Hyun *et al*., 2000). The cells treated with pronase were submitted to microbial adhesion to solvents (MATS) test, hydrophobic interaction chromatography (HIC) and adhesion to polystyrene test.

XPS

X-ray photoelectron spectroscopy (XPS) analysis was performed on a cell film deposited on a glass slide, using a Perkin-Elmer 1257 spectrometer. For analysis, an achromatic Al (Kα) X-ray source operating at 15 kV (300 W) was used, and the spectrometer, calibrated with reference to Ag $3d_{5/2}$ (368.27 eV), was operated in CAE mode with 20 eV pass energy. Data acquisition was performed with a pressure lower than 1.E-6 Pa. Spectral analysis was performed using peak fitting with Gaussian–Lorentzian peak shape and Shirley-type background subtraction.

Contact angle measurement

The samples were prepared by collecting yeast cells on a cellulose triacetate filter (pore diameter, 0.45 μm) to a density of 10^8 cells/mm^2. To establish constant moisture content, the filters with cells were placed in a Petri dish on the surface of a layer of 1% w/v agar in water containing 10% v/v glycerol until the measurement (Busscher *et al*., 1984). Contact angle were measured by the sessile drop technique, on the samples prepared previously, using an apparatus OCA 15 Plus (Dataphysics). The measurements were performed at room temperature using three different liquids with known surface tensions: water, formamide and diiodometane. The angles obtained on the lawns with these three liquids enabled the calculation, according to the Young–Good–Girifalco–Fowkes equation (van Oss *et al*., 1987), of the total surface tension (γ^{tot}) and their components: Lifshitz–van der Waals (γ^{LW}) and Lewis acid–base (γ^{AB}), which in its turn has a positive (γ^+) and a negative (γ^-) component. The values of the free energy of interaction between cells and water (ΔG_{mwm}) were then calculated according to van Oss (1995).

Results

The retention of cells by the polystyrene can be estimated according to the density of cells adhering to the support. Figure 1 show the cells adhering to the polystyrene surface. The test is based on the assumption that there is a correlation between the hydrophobicity of the cells and their adherence to polystyrene, as proposed by Rosenberg (1981). It is possible to notice that the density of adhered cells is very high at pHs 7.0 and 9.0, decreasing to an intermediate density at pH 5.0 and displaying a poor adhesion at pH 3.0.

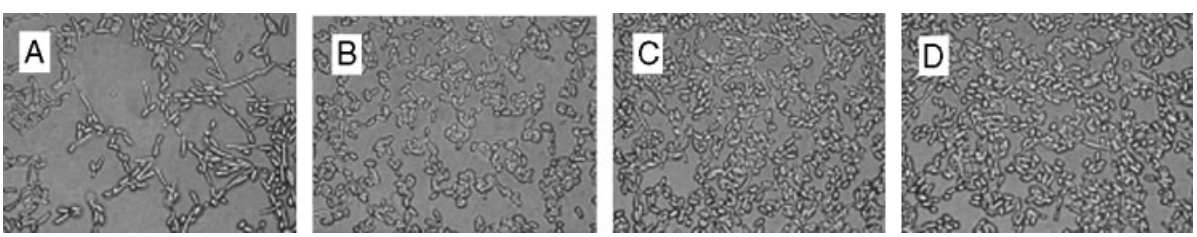

Figure 1. Microscopic (×1000) images of *Y. lipolytica* IMUFRJ cells from the preculture adhered to polystyrene Petri dishes. Cells were suspended in phosphate buffer at pH 3.0 (A), 5.0 (B), 7.0 (C) and 9.0 (D)

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Table 1. Surface characteristics by HIC test for *Y. lipolytica* IMUFRJ cells from the preculture (0 h) and for *Y. lipolytica* W29

		HIC					
Strains (conditions)		**pH 3.0**	**pH 4.0**	**pH 5.0**	**pH 6.0**	**pH 7.0**	**pH 9.0**
Y. lipolytica IMUFRJ (preculture)	R_H, %	99.4	98.9	98.9	98.9	99.8	99.5
	R_L, %	98.4	94.9	94.9	94.9	96.3	96.6
Y. lipolytica W29 (preculture)	R_H, %	89.0	84.3	84.3	84.3	86.0	91.3
	R_L, %	77.7	56.8	56.8	56.8	62.2	65.7

The percentage of *Y. lipolytica*'s (IMUFRJ) retention on the chromatographic gel, R_H (retention of cells in high ionic strength) and R_L (retention of cells in low ionic strength), are presented in Table 1 as a function of pH. The cells are all retained by the gel at high ionic strength ($R_H = 100\%$) without any dependence on the pH value. This is an indication of high hydrophobicity. The retention of cells by lower ionic strength is also very high (above 90%) and not affected by the pH.

Table 2 shows the adhesion of *Y. lipolytica* cells, grown for 48 h in YPD medium (preculture), to various solvent–water interfaces (MATS). The cell surface hydrophobicity (Hyph, expressed as the cell adhesion to hexadecane) of the preculture was high (87%) when compared to other hydrophobic microorganisms, such as *S. thermophilus* (30%) (Bellon-Fontaine *et al*., 1996). It can also be noted that the cell surface displays a significant electron donor character (EDC; expressed as the difference between cell adhesion to chloroform and hexadecane) and a very small electron-accepting character (EAC; expressed as the difference between cell adhesion to ethyl ether and hexane). The MATS tests were carried in cells grown for 170 h in a medium without PFC and with 20% v/v PFC (Table 2). The cell adhesions for both growth conditions were very similar. There is a slightly decrease of hydrophobicity along the fermentation (170 h), compared to the precultured cells, and an increase of the electron donor character.

Table 2. Surface characteristics by MATS test for *Y. lipolytica* IMUFRJ cells from the preculture (0 h) and grown for 170 h with no PFC and with 20% PFC and for *Y. lipolytica* W29

	MATS test		
Strains (conditions)	**Hyph (%)**	**EDC (%)**	**EAC (%)**
Y. lipolytica IMUFRJ (preculture)	86.5 ± 6.9	11.1 ± 0.9	2.7 ± 0.2
Y. lipolytica W29 (preculture)	56.8 ± 2.8	22.8 ± 1.1	-9.6 ± 0.4
Y. lipolytica IMUFRJ (170 h, 0% PFC)	73.7 ± 6.5	19.0 ± 1.1	-0.3 ± 0.0
Y. lipolytica IMUFRJ (170 h, 20% PFC)	76.0 ± 6.1	19.7 ± 1.0	4.3 ± 0.2

HIC and the MATS test were also performed with another *Y. lipolytica* strain, *Y. lipolytica* W29, in order to compare the surface characteristics of different strains, and the results are shown in Tables 1 and 2. The hydrophobic gel, in the presence of high ionic strength, is able to retain more *Y. lipolytica* IMUFRJ cells than *Y. lipolytica* W29, at every pH studied. Even so, this last strain is still highly retained by the gel. Nevertheless, in presence of low ionic strength, which does not induce retention, the percentage of *Y. lipolytica* W29 cells retained is much lower, indicating a less hydrophobic character than *Y. lipolytica* IMUFRJ. By the MATS test, it is also possible to identify a much less hydrophobic character for *Y. lipolytica* W29 cell surface as well as a higher electron donor character, which is corroborated by the results obtained by Aguedo *et al*. (2003). Both strains display a small electron-acceptor character.

The adhesion of microorganisms to hydrocarbons generally increases towards the isoelectric points of the organisms, where the cell surface appears uncharged. Significant adhesion only occurs in the absence of electrostatic repulsion, as observed by van der Mei *et al*. (1995). This is why the adhesion of microorganisms to hydrocarbon may vary with the pH and the buffer of the cell suspension.

Electrokinetic zeta potential measurements were performed on the cells from the preculture, as shown in Figure 2. From these results we could identify that the isoelectric point (IEP) for this microorganism is around 2.4. The IEP of a cell surface is determined by the balance between the charges of the anionic and cationic acid/base groups

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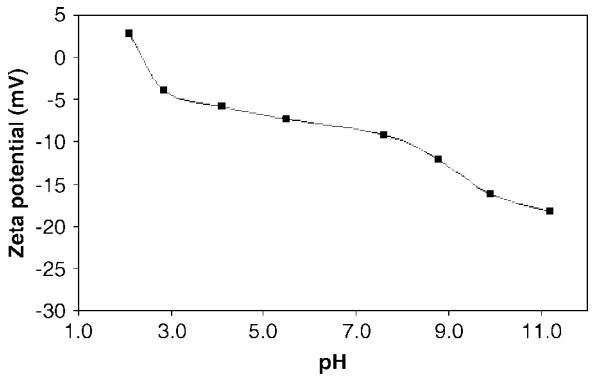

Figure 2. Influence of pH on zeta potential of *Y. lipolytica* IMUFRJ cell suspension

in the cell surface (Rijnaarts *et al.*, 1995). The cell surface is negatively charged at pH values higher than 2.4. For pH values of 3.0–7.0 it displays little change in the surface charge but shows a more pronounced decrease above pH 7.0. A similar zeta potential profile was obtained by Aguedo *et al.* (2005) for *Y. lipolytica* W29, with IEP around 2.5. However, *Y. lipolytica* W29 presents a more negative surface charge (−20 mV) in comparison to *Y. lipolytica* IMUFRJ 50682 (≈ -7 mV) for pH values of 4.0–8.0.

Despite this variation of the cell surface charge with pH, the initial removal rates for *n*-hexadecane and toluene does not vary significantly with the use of different buffers (same pH, $\Delta R_0 < 0.32$) or different pH (same buffer, $\Delta R_0 < 0.34$), as shown in Figure 3. Therefore, the adhesion to hydrocarbon does not seem to be influenced by the surface charge of this microorganism. Taking into account that the values of R_0 were not significantly affected by the pH, a pH value of 7.0 and the PUM buffer were adopted for the MATH test.

The result of the MATH test was in agreement with the MATS test as shown in Figure 4. The hydrophobicity of the preculture cells (0 h), given by the adhesion to *n*-hexadecane in the MATS test (87%) and by the initial microbial removal rate (R_0) of the different hydrocarbons ($R_0 = 0.8 - 1.2$), is high as compared to other hydrophobic cells ($R_0 = 0.4 - 1.2$; van der Mei *et al.*, 1995). Moreover, a slight reduction of the hydrophobicity with fermentation time was also indicated by the MATH test.

To investigate the nature of the interactions between cell surface and hydrophobic compounds, MATS test and HIC were performed after a cell treatment with pronase, which is a mixture of endo-

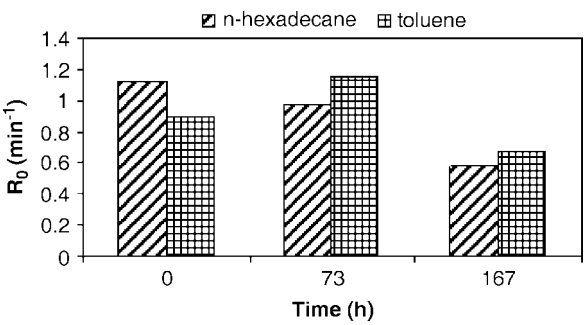

Figure 4. MATH tests carried out on *Y. lipolytica* IMUFRJ cells grown in YPD medium at 250 r.p.m. Initial microbial removal rates (R_0) by *n*-hexadecane and toluene in PUM buffer at pH 7.0

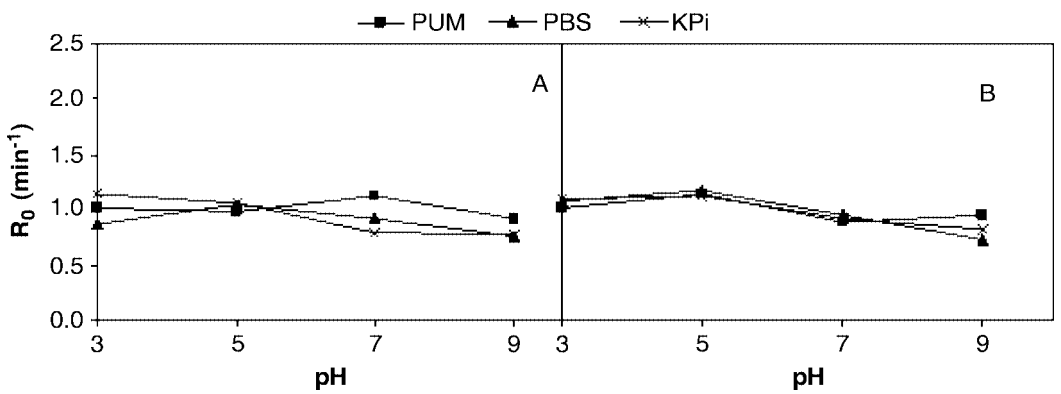

Figure 3. MATH tests carried out on *Y. lipolytica* IMUFRJ cells from the preculture. Initial microbial removal rates (R_0) by *n*-hexadecane (A) and toluene (B) as a function of pH in solutions of different ionic compositions

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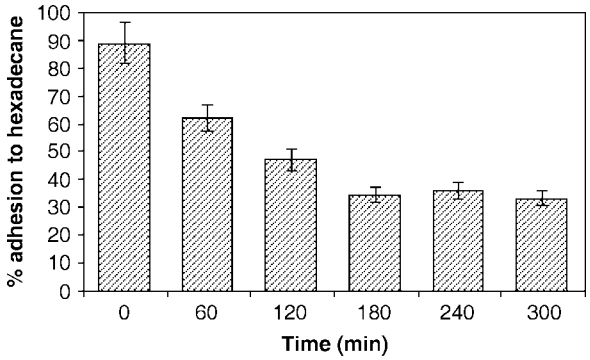

Figure 5. Adhesion to hexadecane carried out with *Y. lipolytica* IMUFRJ cells from the preculture treated with pronase

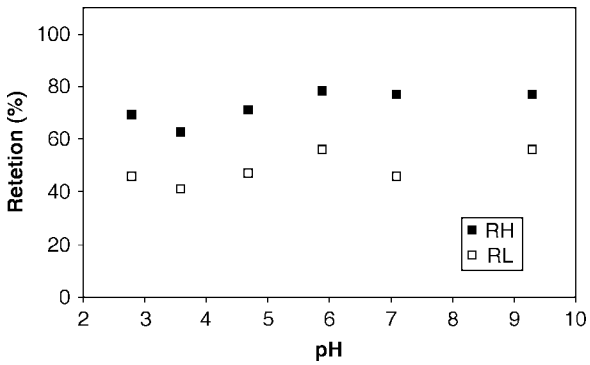

Figure 6. HIC carried out on *Y. lipolytica* IMUFRJ cells from the preculture treated with pronase for 180 min. R_H (retention of cells in high ionic strength) and R_L (retention of cells in low ionic strength)

and exo-proteinases that cleaves almost any peptide bond. The cells' hydrophobicity, determined by the adhesion to hexadecane in MATS test, was significantly reduced after incubation of the cells with pronase for 180 min (Figure 5). This result was supported by the HIC results obtained with cells treated with pronase for 180 min, reported in Figure 6. Even in presence of high ionic strength, which favours cell aggregation, the retention of cells by the hydrophobic gel decreased in comparison to the retention of cells before the treatment (Table 1). Besides, in the presence of low ionic strength (R_L, Figure 6), only ca. 50% of cells were retained by the gel, showing a significant reduction of interactions between cell surface and a hydrophobic surface. This indicates that cell surface characteristics result from some molecule containing protein at the cell surface.

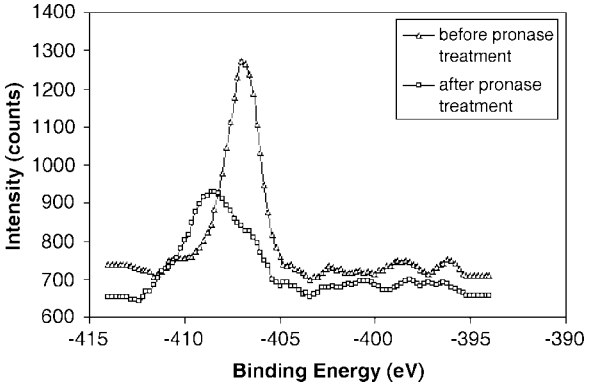

Figure 7. X-ray photoelectron spectroscopy analysis (XPS) of *Y. lipolytica* IMUFRJ cells before and after pronase treatment

To confirm this result, an XPS analysis was performed with the cells before and after pronase treatment. This analysis revealed that the content of cells that were not treated with pronase was 51.0% carbon, 5.9% nitrogen, 41.8% oxygen, and for pronase-treated cells was 49.6% carbon, 3.8% nitrogen, 45.7% oxygen. Not only was a reduction of the nitrogen content observed but also a change on its oxidation state, as can be seen in Figure 7. After pronase treatment the nitrogen peak indicates that it has suffered some chemical modification and that more than one type of nitrogen is present. The shift to higher absolute binding energy values indicates an oxidation of the nitrogen. This analysis indicates that the protein present in the cell surface was degraded. The change in surface behaviour observed after the treatment shows the importance of the protein to the hydrophobic character of the cell.

The cell total surface energy was investigated by the sessile drop technique. Contact angles were measured directly from the images of the solid–liquid meniscus of a liquid drop set on a cell film using a CCD camera. Five separated readings were averaged to obtain one representative value of contact angle for each studied surface and liquid. The liquids used and their parameters are listed in Table 3.

The results for the contact angle measured on the surface of *Y. lipolytica* cells are presented in Table 4. The value of the water contact angle can give preliminary information on the hydrophobicity of cells. For all conditions, the contact angle between water and cell surface is $<50°$ and $>20°$,

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Table 3. Values of the surface energy components of the testing liquids

Testing liquid	γ^{TOT} (mJ/m^2)	γ^{LW} (mJ/m^2)	γ^{AB} (mJ/m^2)	γ^+ (mJ/m^2)	γ^- (mJ/m^2)
Water	72.8	21.8	51.0	25.5	25.5
Formamide	58.0	39.0	19.0	2.28	39.6
Diiodo-methane	50.8	50.8	0	0	0

Table 4. Contact angles for *Y. lipolytica* IMUFRJ cells from the preculture (0 h) and grown for 170 h with no PFC and with 20% PFC and for *Y. lipolytica* W29

Y. lipolytica cells	Water	Diiodomethane	Formamide
IMUFRJ, 0 h	28.0° ± 3.4°	76.9° ± 3.4°	29.6° ± 2.1°
IMUFRJ, 170 h, 0% PFC	26.0° ± 0.7°	62.2° ± 6.6°	34.4° ± 1.4°
IMUFRJ, 170 h, 20% PFC	25.7° ± 0.7°	80.7° ± 1.9°	31.3° ± 3.7°
W29, 0 h	27.4° ± 1.9°	60.1° ± 0.7°	41.0° ± 1.4°

indicating an intermediately hydrophobic character according to the definition of Rijnaarts *et al.* (1995).

The affinity of the cells for the water phase becomes evident with the positive value for the free energy of interaction between cells and water (ΔG_{mwm}), which is given by equation 1 and whose values are listed in Table 5. A negative value for this parameter would indicate a tendency of cell aggregation in aqueous phase (van Oss, 1995), which in fact has not been observed:

$$\Delta G_{mwm} = -2(\sqrt{\gamma_m^{LW}} - \sqrt{\gamma_w^{LW}})^2 - 4(\sqrt{\gamma_m^+\gamma_m^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_m^+\gamma_w^-} - \sqrt{\gamma_m^-\gamma_w^+}) \quad (1)$$

The total surface energy (γ^{TOT}) of the cell film is given by equation 2:

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \quad (2)$$

and, according to Lewis, the acid–base interaction (γ^{AB}) can be decomposed as in equation 3:

$$\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-} \quad (3)$$

From Table 5, it can be observed that the positive Lewis acid–base component (γ^+) is much smaller than the negative one (γ^-), which implies an electron donor character of the cell surface, agreeing with results from the MATS test. van Oss (1997) reported that virtually all biosurfaces are predominantly electron donating as a consequence of the prevalence of oxygen in the Earth's lower atmosphere and the hydration of the microbial cell surface, which agrees with our results. Table 5 also shows that *Y. lipolytica* W29 presents a somewhat more hydrophilic character than *Y. lipolytica* IMUFRJ but not enough to explain the differences observed on the MATH test and HIC.

Discussion

Both the HIC and the adhesion to polystyrene test refer to the retention of cells by a hydrophobic surface. The HIC method also involves the retention of cells in high ionic strengths (R_H). According to Mozes and Rouxhet (1987), this parameter fails to show differences between microorganisms that show different surface properties according to many other methods, i.e. hydrophilic and

Table 5. Surface tension components and free energies of interactions for *Y. lipolytica* IMUFRJ cells from the preculture (0 h) and grown for 170 h with no PFC and with 20% PFC and for *Y. lipolytica* W29

Y. lipolytica cells	γ^{LW} (mJ/m^2)	γ^+ (mJ/m^2)	γ^- (mJ/m^2)	γ^{AB} (mJ/m^2)	γ^{TOT} (mJ/m^2)	ΔG_{mwm} (mJ/m^2)	ΔG_{mwh}[a] (mJ/m^2)
IMUFRJ, 0 h	19.1	6.9	47.8	36.2	55.3	17.9	−5.4
IMUFRJ, 170 h, 0% PFC	27.3	2.1	54.8	21.5	48.7	33.3	−13.2
IMUFRJ, 170 h, 20% PFC	17.1	7.4	51.2	38.9	56.1	19.1	−1.6
W29, 0 h	28.5	0.9	59.9	14.4	42.9	43.5	−15.2

[a] ΔG_{mwh}, free energy of interaction between cells (m) and hexadecane (h) immersed in water (w) calculated by Dupré equation (van Oss, 1995).

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hydrophobic cells may present high R_H. It is only a good parameter in a case of positive hydrophilicity. This may happen because protein molecules of the cell wall dehydrate at high ionic strength through a 'salting-out' effect, reducing their adsorption onto low-energy surfaces (van Oss, 1995). However, at low ionic strength and high pH these proteins restore the original surface hydrophobicity and the electrostatic repulsions prevent the retention by phenyl sepharose (R_L) or polystyrene, except in the case of hydrophobic microorganisms. Thus, by gathering the information of both tests it is possible to identify *Y. lipolytica* IMUFRJ as a hydrophobic microorganism by those assays, as it presents $R_L > 0$ and adhesion to polystyrene in high pH values. The reduced R_L of *Y. lipolytica* W29 cells in comparison to R_H might indicate a hydrophilic character of the surface of this strain.

As mentioned previously, the MATS test is based on the comparison between the affinity of microbial cells for a monopolar solvent (acidic or basic) and an apolar solvent, both having similar van der Waals interactions. Therefore, the results obtained with different pairs of solvent (chloroform/hexadecane and ethyl ether/hexane) indicate that the interactions between *Y. lipolytica* (IMUFRJ 50682) and an organic phase are related mainly to apolar or Lifshitz–van der Waals interactions and not so much to Lewis acid–base interactions.

The results of the MATS and MATH tests are in agreement with the information obtained by HIC and adhesion to polystyrene in what concerns the hydrophobicity of the strain studied. This contrasts with the results obtained to the other strain, *Y. lipolytica* W29, which shows a more hydrophilic surface character. This hydrophilic character of *Y. lipolytica* W29 had also been detected by Aguedo *et al*. (2005), which confirms that the high adhesion of *Y. lipolytica* IMUFRJ for hydrophobic compounds and surfaces is a particularity of this strain.

By definition, hydrophobic compounds, cells, particles or surfaces are those for which $\Delta G_{mwm} < 0$, whereas hydrophilic compounds, cells, particles or surfaces are those which $\Delta G_{mwm} \geq 0$ (van Oss, 1995). As the measurement of contact angles probe exclusively the hydrophobicity of cell surface rather than other physicochemical and structural factors involved in microbial adhesion, it is possible to state from the present results that *Yarrowia lipolytica* IMUFRJ 50682 presents a hydrophilic cell surface. Although these results seem to be inconsistent with the adhesion tests, the negative values of free energy of interaction between cells and hexadecane immersed in water (ΔG_{mwh}) show that the attraction of the cell surface and the hydrocarbon is thermodynamically favoured when they are immersed in water. Indeed, when these cells are directly inoculated in an organic phase, they do not disperse. An aqueous phase is needed for the yeast cells to interact with perfluorocarbons or hydrocarbons. van Oss (1995) has stated that hydrophobic attractions can prevail between one hydrophobic and one hydrophilic site immersed in water because hydrophobic attractions are principally due to the hydrogen-bonding free energy of cohesion of the water molecules of the liquid medium in which the molecules, sites or particles are immersed.

According to contact angle measurements, *Y. lipolytica* W29 and *Y. lipolytica* IMUFRJ show similar surface characters, while having different adhesions to hydrophobic compounds. This shows that, although the contact angle measurement is a good method to determine the hydrophobicity of surfaces, it is important to combine such results with other tests, such as MATH, MATS and HIC, to characterize a cell surface and the cell interaction behaviour.

The low IEP and the negligible effect of pH on the MATH test for *Y. lipolytica* IMUFRJ indicates that the cell surface behaviour is not the result of the direct involvement of proteins in the interactions between cell surface and organic compounds, as the conformational structure of proteins is greatly influenced by the pH and the isoelectric point (IEP) associated with proteins are usually higher due to the presence of NH_2 groups (Rijnaarts *et al*., 1995). The low value of the IEP for *Y. lipolytica* IMUFRJ cell surface indicates that the charges result mainly from COO^- groups and therefore from polysaccharides. However, after the pronase treatment the cell adsorption to hexadecane was substantially reduced. The presence of other lytic enzymes in some commercial preparations of pronase has been reported (Kollar *et al*., 1997). Therefore, other cell wall components might be degraded as well. The XPS analysis shows a reduction on the surface nitrogen and a change on its oxidation. This indicates that proteins from the cell surface were removed by pronase, leaving a different cell surface exposed that seems to

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be less hydrophobic than the original one. These proteins may be glycosylated, forming glycoproteins that would respond by adhesion between hydrophobic compounds and the cell surface. A more detailed study is being carried out to identify these molecules.

Conclusion

The results obtained in the present work demonstrate that the cell surface of *Y. lipolytica* IMUFRJ 50682 is hydrophilic and possesses high attraction to hydrophobic surfaces or molecules when previously immersed in water. The interaction between the cells and the hydrophobic surfaces or molecules is mediated by proteins or glycoproteins of the cell wall. Moreover, the interactions observed between this strain and the non-polar solvents are linked to apolar or Lifshitz–van der Waals interactions. The surfactant secreted by these cells may further enhance this interaction.

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